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Publication date:
1974

Document Version
Publisher's PDF, also known as Version of record

A. E. K. Risø

Risø - M - 1732

Risø - M - 1732

Title and author(s)

Trace Multi-element Analysis of Biological Tissue by Proton-induced X-ray Fluorescence Spectroscopy

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Date July 1974

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Group's own registration number(s)

H/TM 229

pages + tables + illustrations

Abstract

A development of sample preparation technique for trace multi-element analysis of biological tissue by proton-induced, X-ray fluorescence spectroscopy is described. The samples, which were sections of liver tissue from rats and guinea pigs, were found to be representative of the liver with respect to element content.

More than 20 elements were simultaneously determined in the liver samples, and the detection limits for the heavy elements, i.a. Cr, Ni, As, Se, Sr, Mo, Cd, and Pb, were 0.2-2 ppm in dry matter.

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FI 25-204

ISBN 87 550 0276 5

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1. INTRODUCTION

Trace element analysis of biological tissue is of increasing interest in connection with investigations of environmental pollution. Such studies often require multi-element determinations with extremely low limits of detection. Multi-element analysis can be accomplished by many instrumental techniques: Atomic absorption, flame photometry, neutron activation, X-ray fluorescence, emission spectrophotometry, etc.[1,2]. If several elements have to be determined in a sample to low limits a combination of techniques may normally be required.

Proton-induced X-ray fluorescence analyses have, however, proved to be a method for simultaneous determination of the elements with atomic number $>$ 14 in quantities down to 10^{-13} g and in concentrations down to 0.1 ppm. Since 1971 this method has been used at the Niels Bohr Institute in Copenhagen for multi-element analysis of air-borne particulate matter. Preliminary experiments with the method applied to biological tissue showed that it is suitable for determination of many elements in concentrations of interest. However, the experiments also indicated that improvement of the preparation technique was needed.

The present report describes further developments of the technique of sample preparation. The investigations were carried out on liver tissue - partly because high concentrations of e.g. heavy metals are to be found in this organ and partly because liver tissue samples of the shape needed for the analysis were expected to be representative of the liver. Liver from rats and guinea pigs was used.

The accuracy of the analytical method was expected to be better than $\pm$5% in favourable cases, but it was not known in advance whether the thickness of the sample could be reproduced with a corresponding accuracy. Therefore it was decided to examine the possibilities of measuring the sample thickness sufficiently accurately by means of protons scattered from atomic nuclei of the sample.

The aims of the investigations were the following:

1) To develop a technique for preparing samples of biological tissue suitable for trace element analysis by proton-induced X-ray fluorescence spectroscopy.
2) To develop an improved technique for determination of the total quantity of tissue in the sample with the purpose of determining the concentration of elements in dry matter.

3) To determine the limits of detection for the elements of interest from a biological point of view.
4) To establish whether the concentrations of the elements vary significantly from sample to sample within the same organ.

2. PRINCIPLES OF PROTON-INDUCED X-RAY FLUORESCENCE SPECTROSCOPY

The physical fundamentals and the experimental problems of proton-induced X-ray fluorescence spectroscopy are described in this section with special attention to the properties of the samples. A more detailed description of the physical fundamentals can be found in ref. 3. The principles of the experimental set-up appear from figure 1:

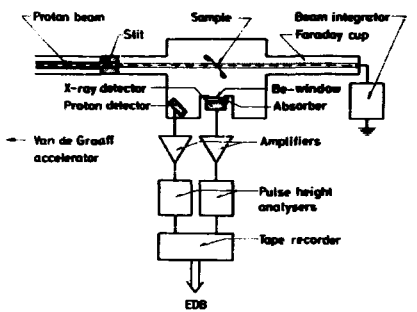

Figure 1. Principles of the apparatus for proton-induced, X-ray fluorescence analysis.

A well defined beam of protons with high energy (2-3 MeV) is injected into the sample causing electrons to be ejected from the inner electron shell of the atoms, e.g. the K- and L-shells. The holes in the inner shells are subsequently filled by electrons, and X-radiation with characteristic energy is emitted. The energies of the X-ray quanta are typical for the element and the intensities indicate the quantity of the element from which they are

emitted. The X-ray spectrum is measured by a high resolution detector and the integrated proton beam by a Faraday cup.

2.1. Production of X-ray Radiation

In an infinitely thin sample, an element with atomic number Z give rise to a number of X-ray counts given by[3]:

$$n_i = K \times N_z \times N_p \times \epsilon_i^{emis} \times \epsilon_d \times C_{abs} \qquad (1)$$

K: Proportionality constant,

N_z: The number of atoms of element Z,

N_p: The number of incident protons,

ϵ_i^{emis}: The emission cross section which characterizes the efficiency of the protons in producing X-radiation in the element in question,

ϵ_d: The efficiency of the detector,

C_{abs}: The absorption reduction factor accounting for the attenuation of the X-rays on their way from the sample to the detector.

For a given shell the energy of the X-ray quanta emitted by electron transitions increases with increasing atomic number. In addition, for a specific element the X-radiation energy corresponding to transition to L-shells is less than that corresponding to transition to K-shells. Due to limited relative energy resolution of the detector at low X-ray energies and small C_{abs} at small Z, the determination of the content of elements with low atomic numbers is impossible. In practice, the X-ray emission from transitions to K-shells can only be used if the atomic number is above 14, and X-rays from transitions to L-shells if it is above 40. On the other hand, the emission cross section decreases with increasing atomic number and is greatest for L-lines at a specific atomic number. Consequently the K-lines are suitable for determination of elements with atomic numbers between 14 and ~ 50, whereas L-lines are more suitable for atomic numbers greater than ~ 50.

For some elements the K- and L-lines overlap. However, for each shell the characteristic X-rays are split into sub-shells, e.g. the transitions to K-shells into K_α and K_β and L-shell transitions into L_α, L_β and L_γ. These double- and triple-lines are used to distinguish between such

elements. Nevertheless, this complication, coupled with the finite energy resolution of the detector, limits the possibilities of determining small quantities of some elements when certain other elements are present in large quantities.

In the case of thick samples the absorption of X-rays in the matrix may be essential, especially for elements with low atomic numbers. If the surface of the sample is flat a correction is possible. Thin samples are, however, desirable due to uncertainties of the correction factor for very thick samples. The energy loss of the protons in thick samples may also necessitate correction factors. In the following, a sample which is thicker than the stopping range of the protons is termed "∞-thick". Stopping ranges of protons are given in ref. 5.

2.2. Background Radiation

During analysis the trace element is normally embedded in a matrix which gives rise to a background radiation. The stopping of electrons ejected from the matrix gives rise to the emission of continuous background radiation. Thereby the sensitivity of concentration is limited to 0.1 - 1 ppm. As seen in figure 3, the background radiation is most important at low X-ray energies. Due to the previously mentioned phenomenon it is important that the quantity of matrix material is as small as possible. However, compared to conventional X-ray fluorescence this method is superior due to the much lower background radiation.

2.3. Other Factors Affecting the Sensitivity Obtainable

Heating of the sample due to deceleration of protons, in combination with its presence in vacuum, may be a problem if volatile compounds are present. It is therefore important to limit the heating by appropriate sample preparation and mounting and by using proton beams of low intensity.

Samples with poor electrical conductivity may become charged during the proton irradiation. Electrons floating around in the scattering chamber may thus be accelerated against the sample, causing emission of continuous background radiation.

3. EXPERIMENTAL

3.1. Preparation of Tissue Sections

Tissue sections of the following four types were investigated:

a) 2 mm hand-cut sections. The sections are cut from semi-frozen tissue using a scalpel, then placed on the sample holder and freeze dried. The thicknesses of these samples could not be reproduced and the surfaces were irregular. In addition, adhesion to the sample holders was poor.

b) 60 µm self-supporting, freeze-dried cryostat sections. After being cut the sections are placed in petri dishes. Each section is covered with a light plastic disc to prevent it from curling up while drying. The main problem is to allow the sections to shrink without curling up during drying. 50-70% of the sections crack during mounting and during the final drying in an exicator. The sections remain in the cryostat for two days at -8°C. Afterwards they are mounted on the sample holders and the final drying takes place in an evacuated exicator with silica gel at room temperature.

c) 10 µm sections embedded in resin. A piece of tissue (approx. 6x6x3 mm) is freeze dried and embedded in resin in vacuum. After polymerisation (18 hours at 60°C) the 10 µm sections are cut on an ultratome.

Preparation by this technique is very time-consuming. In addition, It is very hard to cut this size of section using an ultratome.

d) 20 µm cryostat sections placed on 0.5 µm polystyrene backings. The sections are dried in an exicator as described in b). These sections curl up during dehydration, causing the polystyrene films to break.

The sections a), b) and c) were placed on sample holders of the type shown in figure 2. The aluminium back of the sample holder ensures discharging of the sample and the plastic front of the sample holder prevents scattered protons from generation of characteristic X-ray emission in the aluminium and in the impurities of the aluminium.

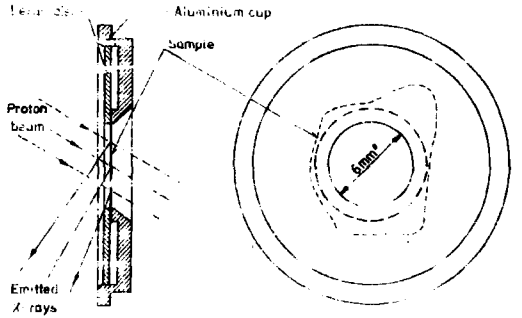

Figure 2 Sample holder for tin.ne sections for proton induced, X-ray fluorescence analysis.

3.2. Analysis

In the actual experiments the high energy protons were generated by the 4 MeV Van de Graaff accelerator at the Niels Bohr Institute, Copenhagen. The X-ray spectra were measured by a 30 mm^2 liquid-nitrogen-cooled Si(Li)-detector (Kevex mark AA). The spectra were recorded by a Nuclear Data 2200 pulse height analyser and stored on magnetic tape for later analysis on a Univac 1110 computer.

In order to obtain the best possible sensitivity for a large number of elements, each sample was thrice irradiated with protons under different conditions. A typical set of the three spectra is shown in figure 3.

Elements with atomic numbers $Z \leq 20$ were determined by irradiation with 2 MeV protons. Elements with $20 \leq Z \leq 30$ were determined by irradiation with 2 MeV protons and with an Al-absorber between sample and detector. The absorber attenuates the X-radiation from the elements P, S, Cl, and K which are present in high concentrations. Hereby the dead-time losses are reduced and the sensitivity is improved. Finally the elements with $Z \geq 30$ are determined by irradiation with 3 MeV protons and an Al-absorber which drastically reduces the radiation from elements with $Z \leq 28$. By using higher energy the yield of the X-ray emission was increased, especially for heavy elements. However, also the radiation from the matrix was increased; therefore the net result is improved sensitivity for heavy

elements only. Typical times for the three irradiations were 2, 8 and 8 minutes respectively and the collected charges were 0.02, 10 and 40 microcoulombs.

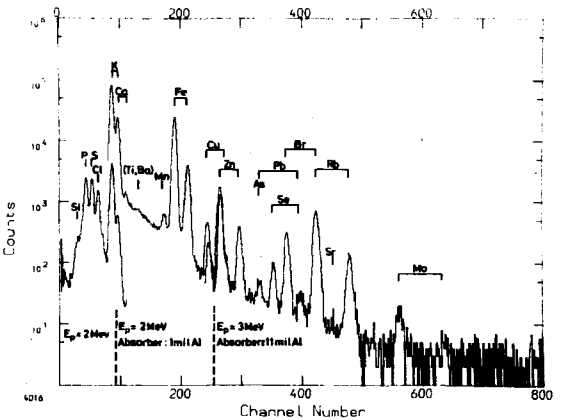

Figure 3. A typical set of X-ray spectra. The spectra are directly written out by the computer. Afterwards some of the peaks are indicated by lines and symbols of the elements.

The thickness of the sample was measured during the last irradiation by recording an energy spectrum of the protons, which were elastically scattered from the atomic nuclei in the sample, at an angle of 135°. Protons scattered near the back of the sample suffered an energy loss depending on the path length in the sample, while protons scattered near the front lose only a small fraction of their energy. Accordingly the width of the peak in the proton spectrum gives a measurement of the sample thickness. A completely homogeneous sample results in a box-shaped peak. The slope of the low energy tail of the peak shows the degree of irregularity. The width of half maximum is used for determination of a mean thickness.

Alternatively, the thicknesses might be determined by comparing the elemental content in several sections from one organ with the concentrations found from measurement on an " ∞-thick" sample from the same organ. The effective thickness of the 2 mm sections is the same as that of a sample having a thickness equal to the stopping length of the protons. Since this is known[5], it is possible to determine the concentrations in the 2 mm samples.

4. RESULTS AND DISCUSSION

In order to choose the most suitable type of preparation and subsequently to investigate this in greater detail the following two series of measurements were performed:

I. Analysis of the four different types of sections from guinea pig liver.

II. Analysis of a total of approx. 30 liver sections from 8 different rats which had lived under the same conditions.

The purpose of the first series was to find the most suitable type of section, i.e. one giving the lowest detection limits and at the same time possessing a reasonable mechanical stability.

The second series of measurement was performed on the most suitable type of section in order to obtain information about the reproducibility of the method and the variation in the elemental content within one organ and within one group of animals. The possibilities for determination of the section thickness from measurement of "∞-thick" sections and scattered protons were considered.

The results of the first series, which are found in table 1, were converted into ppm in dry tissue, assuming that the sections had the quoted thickness, a density of 1 g/cm^3, and an original water content of 70%[4].

In the second series of measurements, one 2 mm section and 9-10 60 µm self-supporting sections from each animal were analysed. Results are found in table 2, 3 and 4. The amounts in table 2 are given in µg/cm^2. The measured number of X-ray quanta is assumed to be directly proportional to the number of atoms per square unit (cf. sect. 2.1.); this number is easily converted to µg/cm^2. If the section thickness is known, it is possible to find the concentrations in the tissue material from the absolute amounts. The mean values of the elemental content in each animal were found and the standard deviation of the results for one animal were calculated from the scatter of the experimental results. Besides the elements mentioned in the tables, Ni, Sr and Pb were found in concentrations from 0.5 to 3 ppm in several sections.

4.1. Choice of Preparation Method

Four types of preparation (cf. sect. 3.1.) were investigated and the results are found in table 1.

a) 2 mm sections. Even if known not to be ideal (cf. sect. 2.1.), these preparations are used because it is possible to determine the concentrations after correction due to absorption (cf. sect. 3.2.). The corrections are largest for the lightest elements. For elements with Z $\leq$25 the uncertainty is very high ($\pm$ 20%). The analyses gave rise to several difficulties, e.g. charging of the surface during bombardment, and intense heating (the protons deposit the whole energy in the sample) followed by partial collapse and evaporation of volatile compounds.

b) 60 µm self-supporting sections. The values for the detection limits of these sections are similar for those of the 2 mm samples. The mechanical strength was sufficiently good. The thickness makes an absorption correction necessary, but this is moderate.

c) 10 µm embedded sections. The detection limits are about a factor 3 higher than for a) and b) sections, due to the larger amount of matter in the background matrix. The high content of elements such as Cl, K, Ca, and Fe probably originates from impurities in the resin. Consequently it is necessary to improve the preparation technique if these sections are to be used. It is possible that volatile matter (e.g. Hg-compounds), which would otherwise escape during evacuation and bombardment, is retained in these samples.

d) 20 µm sections on backing. The lack of strength made it difficult to measure these samples, and for this reason they are unsuitable for routine analysis.

Both with respect to preparation and analysis, the 60 µm self-supporting sections are definitely the most suitable of those investigated. Using 10-20 µm sections of same type it might be possible to achieve approx. 25% lower detection limits for elements having Z $\leq$25. It was, however, impossible to prepare such thin sections (cf. sect. 3.1.). The 60 µm self-supporting sections are therefore used in the second series of measurements mentioned above.

4.2. Detection Limits

The detection limit is defined as the concentration which is determined with an uncertainty of 50%, partly due to the statistical uncertainty of the background counts at the position on the spectrum where the peak of the characteristic X-ray is found (cf. figure 3) and partly due to the experimental uncertainty. The detection limits are determined by the recorded spectra.

The detection limits appear in part from table 1. The second series of measurements mainly confirmed these values. The following summarizes the detection limits obtained:

$Z \leq 13$	(Al)			not detectable
(Si)	14 $\leq Z \leq$	21	(Sc)	200-20 ppm in dry tissue[a]
(Ti)	22 $\leq Z \leq$	38	(Sr)	20-0.5
(Sr)	38 $\leq Z \leq$	56	(Ba)	0.5-8
(Ba)	56 $\leq Z \leq$	79	(Au)	8-(20[a,b])
(Hg)	80 $\leq Z$			2

a) spectral interference from elements which are always found in high concentrations are taken into account.

b) estimated value.

Since spectral interference cannot be excluded, the above values must be taken with a certain reservation. A larger amount of one element might hide the presence of another, e.g. the detection limit for Co is 5-10 ppm caused by the high Fe content, and the limit for As is roughly 10% of the Pb content in samples with much Pb, and approx. 50% when the Pb content is near the detection limit.

4.3. Measurement of Thickness and Calculation of Concentration

The section thickness can in principle (cf. sect. 3.2.) be determined from the width of the peak of the proton spectra. This determination is not good for several reasons. E.g. the probability for scattering of protons alters while they are slowed down on their way through the sample, and the finite energy resolution of the detector tends to broaden the peak. It is however, possible to determine the relative thickness by comparing the spectra from sections of the same type. Fig. 4 shows spectra from the two 60 µm sections investigated in the first series of measurements. It is known[5] that the energy loss in 1 µm of tissue is approx. 11 keV. The difference in width corresponds to a difference in energy loss of 81 keV (2 1/2 channel), i.e. a difference in thickness of 7 µm.

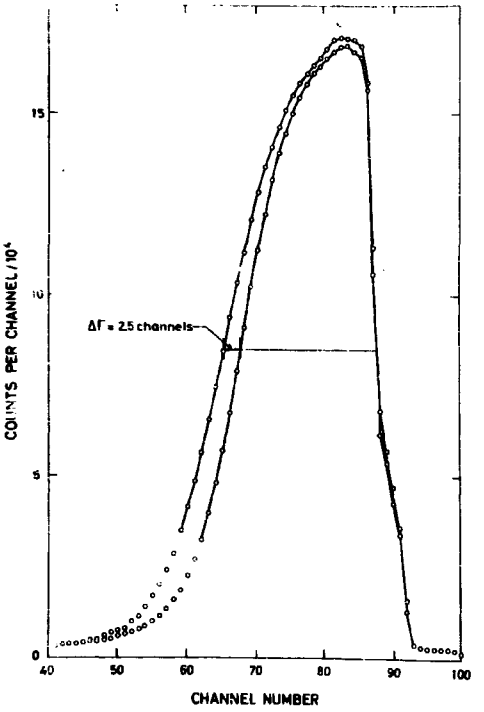

Figure 4. Proton energy spectra for two tissue sections. The peak widths at half maximum are measurements of the section thicknesses. ΔΓ is the difference in peak width of the two samples. The slope of the low energy tail of the peaks shows the degree of irregularity.

The alternative thickness determination (cf. sect. 3.2.) is carried out by comparing the amounts of Fe, Cu, Zn, and Rb in the 60 µm sections from animals 1, 2, 4 and 5, in the second series of measurements, with the concentrations found from the measurements of "∞-thick" samples from the same organs (tables 2 and 4). Hereby the mean thickness of the 60 µm sections from these animals was determined to 1.7 mg/cm^3. Assuming that the density of the dry tissue is 1 g/cm^3 and that 70% of the

"wet" tissue is water, this corresponds to a thickness of 57 μm.

Using the above mean thickness, the concentrations are calculated (and listed in table 3) from the absolute values in table 2 and the results of the proton analysis.

For the most accurately determined elements the standard deviations (DV in table 2) are 3-9%. The deviations originate from the experimental uncertainties and the variation of the elemental content and the section thickness. Taking the two first contributions into account, the last is estimated to be $\leq \pm 5\%$, while the deviations in the calculated concentrations are $\leq \pm 10\%$. The difference in section thickness is apparently less than the uncertainty of the determination of the relative thicknesses. This uncertainty is probably due to the presence of small fractures in several of the sections. The proton measurement gives the mean thickness exclusive fractures, while the elemental content is determined in the irradiated area as a whole.

In this case the measurement of scattered protons seems unnecessary. It might, however, be reasonable to check the thickness in this way for control. Relatively little work is involved in collection and analysis of the proton data.

4.4. Concentration Variations in Liver Tissue from Rats

The investigations of concentration variations were based on the results from analyses of approx. 80 liver sections from the eight rats (cf. tables 2 and 3). No significant differences were observed in the elemental content in different sections from the same organ. Therefore it appears to be sufficient to analyse a couple of samples from each organ, even if only approx. 0.2 mg of dry tissue is used for the analysis.

For the majority of the elements no significant differences in elemental content were found in animals which had lived under the same conditions. Significant variations were only detected for Cl, Fe, Se, and As. It deserves notice that the variations in the content of Fe, As, Se, and possibly Mo are correlated. As an illustration, the contents of K, Fe, and Se are shown in fig. 5.

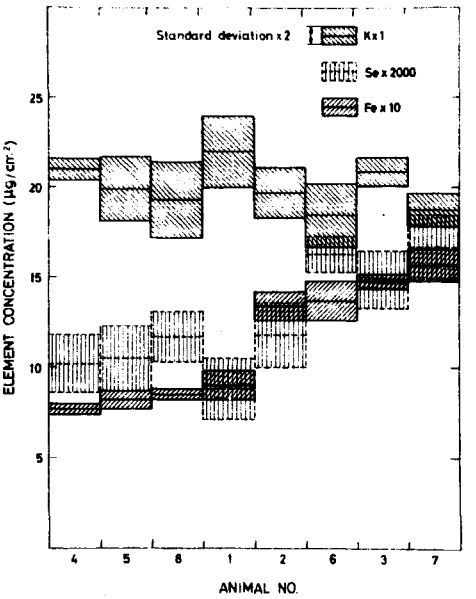

Figure 5. Contents of K, Fe and Se in liver from the eight rats investigated, arranged in order of increasing Fe-concentration.

5. CONCLUSION

Of the preparations investigated the 60 μm self-supporting freeze-dried sections proved to be the most suitable, both from the preparing and the analytical point of view. The detection limits were found to be 0.5-2 ppm in dry tissue for some of the most interesting elements. Using proton-induced X-ray fluorescence spectroscopy it is hardly possible to obtain essentially better detection limits with other types of section.

The section thickness could be determined within ± 10% from measurements on "∞-thick" sections and elastic scattered protons. Statistical considerations showed that the variation in thickness of the 60 µm self-supporting freeze-dried sections was less than ± 5%. In this case the proton measurements could seem superfluous, but they were useful for control.

Even if the individual analyses are based on only approx. 0.2 mg dry tissue, it seems sufficient to analyse a few sections from each liver. For some elements the concentration variations from animal to animal proved to be correlated.

ACKNOWLEDGEMENTS

Thanks are due to the Danish Agricultural and Veterinary Research Council for the financial support of the project, and to the Niels Bohr Institute, Copenhagen for placing the Van de Graaff accelerator at our disposal for the investigations.

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Table 1

Concentrations and detection limits of elements in guinea pig liver samples prepared in four different ways, a, b, c, and d (cf. sect. 3.1.). The detection limit of As (x) is closely related to the Pb content (cf. sect. 4.2.).

Element	a		b			c			d		
	Conc. (ppm)	Det. Lim. (ppm)	Conc. (ppm)		Det. Lim. (ppm)	Conc. (ppm)		Det. Lim. (ppm)	Conc. (ppm)		Det. Lim. (ppm)
P	7300		7000	10500		24000	22000		7100	7100	
S	1900		2500	3500		2800	4800		1800	2100	
Cl	1600		3700	4800		21000	33000		3000	3000	
K	10000		8700	11000		25000	22000		10000	13000	
Ca	134		290	230		600	600		225	300	
Ti	8	4	5		3		15	12			6
Cr	2	2			3			10			5
Mn	24		14	15		45	33		14	14	
Fe	580		480	580		1400	1300		516	690	
Ni	2.4	2	3.5	6.5	2		7.5	4.5	3	12	2
Cu	250		160	150		300	260		193	225	
Zn	200		130	130		225	210		140	146	
As	1.1	(x)	1	0.5	(x)	1	2	(x)			(x)
Se	0.2	0.2	1		0.5			1.5			1
Br	23		37	38		51	45		36	54	
Rb	78		50	55		102	90		41	60	
Sr		0.3	1	1	0.5	3	2	1.5		3	0.5
Mo	6	1	4	4	1	6	6	3	3	4	1.5
Cd	7	2			1		20	6	6	6	2
Ba		12			8			45			15
Pb	2	2	5	4	3		6	4			3

Table 2

The mean values of the contents of elements in 60 μm self supporting freeze-dried liver sections from the eight rats investigated. UC denotes the mean values of the experimental uncertainties in %, including systematical experimental errors estimated to 10%. DV denotes the standard deviations of the elemental contents in sections from the same animal.

Element	1			2			3			4		
	Conc. ($\mu g/cm^2$)	UC (%)	DV (%)	Conc. ($\mu g/cm^2$)	UC (%)	DV (%)	Conc. ($\mu g/cm^2$)	UC (%)	DV (%)	Conc. ($\mu g/cm^2$)	UC (%)	DV (%)
P	22.7	10	9	21.6	10	9	22.0	10	10	20.9	10	6
S	10.4	10	12	10.1	10	9	9.04	10	20	10.9	10	5
Cl	7.58	11	7	7.78	11	12	6.21	11	7	5.24	11	6
K	22.0	11	9	19.7	11	7	20.9	11	4	21.0	11	3
Ca	0.39	20	17	0.42	20	17	0.37	20	12	0.42	19	5
Mn	0.0159	17	19	0.0118	18	15	0.0127	18	17	0.0131	17	12
Fe	0.90	10	9	1.34	10	6	1.48	10	3	0.77	10	3
Cu	0.034	13	16	0.0396	12	15	0.049	13	11	0.041	12	13
Zn	0.290	12	13	0.224	12	7	0.295	12	6	0.290	12	5
As	0.0045	25	20	0.0055	24	21	0.0051	25	17	0.0031	27	34
Se	0.0044	23	19	0.0059	19	15	0.0075	18	11	0.0051	21	16
Br	0.046	14	15	0.048	14	8	0.047	14	7	0.041	14	9
Rb	0.123	12	15	0.121	12	8	0.144	12	8	0.129	12	7
Mo	0.0081	22	22	0.0079	21	20	0.0105	19	14	0.0085	21	18

Table 2. Continued

Element	5			6			7			8		
	Conc. ($\mu g/cm^2$)	UC (%)	DV (%)	Conc. ($\mu g/cm^2$)	UC (%)	DV (%)	Conc. ($\mu g/cm^2$)	UC (%)	DV (%)	Conc. ($\mu g/cm^2$)	UC (%)	DV (%)
P	19.5	11	11	22.5	10	5	21.7	10	5	21.6	10	[illegible]
S	11.4	10	11	10.9	10	4	9.61	11	5	9.38	11	[illegible]
Cl	4.71	11	6	5.89	11	5	5.77	11	5	5.39	11	[illegible]
K	19.9	11	9	18.5	11	10	18.8	11	5	19.3	11	13
Ca	0.41	20	6	0.33	20	13	0.33	18	9	0.43	20	13
Mn	0.0111	19	19	0.0116	18	13	0.0148	16	14	0.0151	17	10
Fe	0.82	10	6	1.37	10	8	1.57	10	6	0.65	10	4
Cu	0.04	13	36	0.032	13	10	0.041	12	12	0.034	13	6
Zn	0.277	12	10	0.245	12	12	0.254	12	8	0.260	12	2
As	0.0033	28	29	0.0057	24	19	0.0043	25	20	0.0030	28	20
Se	0.0053	20	17	0.0082	16	6	0.0082	17	11	0.0059	19	12
Br	0.038	14	7	0.042	14	10	0.043	14	7	0.041	14	5
Rb	0.123	12	12	0.113	12	17	0.132	12	13	0.114	12	4
Mo	0.0067	23	17	0.0093	20	20	0.0100	19	24	0.0060	24	15

Table 3

The results from table 2 with the elemental contents converted to ppm in dry matter.

Element	1			2			3			4		
	Conc. (ppm)	UC (%)	DV (%)	Conc. (ppm)	UC (%)	DV (%)	Conc. (ppm)	UC (%)	DV (%)	Conc. (ppm)	UC (%)	DV (%)
P	13040	10	12	12080	10	10	11597	10	12	11370	10	3
S	5934	10	13	5649	10	14	4729	10	21	6188	10	8
Cl	4348	11	10	4341	11	11	3274	11	11	2976	11	4
K	12610	11	11	11007	11	9	10983	11	9	11920	11	4
Ca	226	20	22	235	20	17	194	20	12	240	19	8
Mn	7.5	17	25	6.6	18	15	6.7	18	21	7.4	17	12
Fe	517	10	11	752	10	9	776	10	6	441	10	5
Cu	19.6	13	26	22.0	12	9	25.5	13	12	23.3	12	17
Zn	166	12	14	125	12	10	155	12	8	165	12	3
As	2.6	25	22	3.0	24	20	2.7	25	21	1.8	27	36
Se	2.5	23	20	3.3	19	15	3.9	18	15	2.9	21	13
Br	26.1	14	17	26.8	14	10	24.6	14	12	23.2	14	6
Rb	70.4	12	16	67.8	12	11	75.5	12	11	73.4	12	3
Mo	4.6	22	21	4.4	21	21	5.5	19	12	4.9	21	21

Table 3. Continued

Element	5 Conc. (ppm)	5 UC (%)	5 DL (%)	6 Conc. (ppm)	6 UC (%)	6 DL (%)	7 Conc. (ppm)	7 UC (%)	7 DL (%)	8 Conc. (ppm)	8 UC (%)	8 DL (%)
P	11070	11	9	13900	10	10	12838	10	12	12158	10	9
S	6476	10	10	6720	10	12	5635	11	12	6896	11	7
Cl	2668	11	9	3630	11	10	3407	11	11	3579	11	9
K	7376	11	7	11311	11	7	11044	11	7	11768	11	14
Ca	213	20	9	208	20	11	194	18	11	281	20	16
Mn	6.1	19	16	7.1	15	17	4.6	17	17	9.3	17	6
Fe	477	10	16	955	10	7	924	11	7	517	10	9
Cu	25.1	13	25	20.3	13	11	24.2	12	7	20.9	13	11
Zn	138	12	9	150	12	7	149	12	7	158	12	4
Y	1.9	26	34	3.1	24	18	2.7	25	14	1.8	26	16
Sr	3.6	20	24	5.1	16	14	4.9	17	11	3.6	19	12
Rb	21.5	14	10	25.8	14	11	25.1	14	7	24.9	14	4
Rh	69.9	12	9	68.4	12	6	77.0	12	9	69.2	12	6
Mo	3.8	23	15	5.6	20	12	5.8	19	19	3.7	24	14

Table 4

The concentrations of elements in dry matter in 2 mm liver sections from the eight rats investigated.

Element	Concentration (ppm)							
	1	2	3	4	5	6	7	8
P	6800	9700	4900	13000	13000	11000	12000	9800
S	800	2100	1600	1800	1100	2500	3100	2500
Cl	1200	1800	1300	1500	700	2300	2600	2300
K	7300	7600	4200	11000	8600	8600	8500	8500
Ca	395	460	286	680	640	630	430	585
Mn	8.6	8.0	4.4	6.8	-	6.8	8.2	8.4
Fe	575	881	620	440	650	998	631	413
Cu	23	20	14	22	9.2	36	26	20
Zn	129	140	84	179	160	161	147	181
Rb	65	75	43	93	60	73	80	89
Mo	3.3	3.9	1.6	3.4	3.2	-	-	-